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Abstract: Intravenous colistimethate sodium (CMS) is used to treat infections with multi-resistant Gram-negative bacteria. Optimal dosing in patients undergoing continuous renal replacement therapy (CRRT) is unclear. In a prospective study, we determined CMS and colistin pharmacokinetics in 10 critically ill patients requiring CRRT (8 underwent continuous venovenous hemofiltration (CVVHD), median blood flow 100 ml/min). Intensive sampling was performed on treatment day 1, 3 and 5 after a 9 MU intravenous CMS loading dose (6 MU if body weight < 60 kg) with a consecutive 8-hourly 3 MU (respectively 2 MU) maintenance dose. CMS and colistin were determined by liquid chromatography with mass spectroscopy. A model-based population pharmacokinetic analysis incorporating CRRT settings was applied to the observations. Sequential model building indicated a monocompartmental distribution for both CMS and colistin, with interindividual variability in both volume and clearance. Hematocrit was shown to affect the efficacy of drug transfer across the filter. CRRT clearance accounted for on average 41% of total CMS and 28% of total colistin clearance, confirming enhanced elimination of colistin compared to normal renal function. Target colistin steady state trough concentrations of at least 2.5 mg/L were achieved in all patients receiving 3 MU 8-hourly. A loading dose of 9 MU followed after 8 h by a maintenance dosage of 3 MU 8 hourly independent of body weight is expected to achieve therapeutic colistin concentrations in patients undergoing CVVHD using low blood flows. Colistin therapeutic drug monitoring might help to further ensure optimal dosing in individual patients.

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Running head: Colistin pharmacokinetics during hemofiltration

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Abstract

Background: Intravenous colistimethate sodium (CMS) is used to treat infections with multi-resistant Gram-negative bacteria. Optimal dosing in patients undergoing continuous renal replacement therapy (CRRT) is unclear.

Materials/methods: In a prospective study, we determined CMS and colistin pharmacokinetics in 10 critically ill patients requiring CRRT (8 underwent continuous venovenous hemofiltration (CVVHD), median blood flow 100 ml/min). Intensive sampling was performed on treatment day 1, 3 and 5 after a 9 MU intravenous CMS loading dose (6 MU if body weight < 60 kg) with a consecutive 8-hourly 3 MU (respectively 2 MU) maintenance dose. CMS and colistin were determined by liquid chromatography with mass spectroscopy. A model-based population pharmacokinetic analysis incorporating CRRT settings was applied to the observations.

Results: Sequential model building indicated a monocompartmental distribution for both CMS and colistin, with interindividual variability in both volume and clearance. Hematocrit was shown to affect the efficacy of drug transfer across the filter. CRRT clearance accounted for on average 41% of total CMS and 28% of total colistin clearance, confirming enhanced elimination of colistin compared to normal renal function. Target colistin steady state trough concentrations of at least 2.5 mg/L were achieved in all patients receiving 3 MU 8-hourly.

Conclusion: A loading dose of 9 MU followed after 8 h by a maintenance dosage of 3 MU 8 hourly independent of body weight is expected to achieve therapeutic colistin concentrations in patients undergoing CVVHD using low blood flows. Colistin

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Antimicrobial Agents and Chemotherapy

Introduction

Colistin is a polymyxin antibiotic with concentration dependent bactericidal properties against a variety of Gram-negative species including *Pseudomonas aeruginosa, Klebsiella spp., Enterobacter spp.* and *Acinetobacter spp.*. Colistimethate sodium (CMS) is the inactive prodrug of colistin, bearing methanesulfonate residues linked to its five amine radicals. It is commercially available as Colistin® (1) and is usually used to treat multidrug-resistant strains. After intravenous administration, CMS is partially hydrolyzed (30-40%) into the active form colistin and to sulfomethylated derivatives, while two-thirds of the prodrug dose are renally excreted unchanged by glomerular filtration (2, 3). Colistin`s bioavailability is therefore limited by what could be considered a type of “renal first-pass effect”. Renal clearance of CMS in healthy volunteers approximates 100 ml/min (4). Conversely, the renal excretion of colistin itself is modest, due to active tubular reabsorption (3). In critically ill patients, Karaiskos and colleagues found the renal clearance of colistin to be not significantly different from zero (5). In patients with renal impairment, a larger fraction of CMS is available for transformation into colistin and both agents accumulate (6, 7), so that a significant dosage reduction is needed (8). The two major dose-dependent toxic effects of colistin are nephrotoxicity due to acute tubular necrosis and neurotoxicity (9).

Critically ill patients may develop acute or acute-on-chronic renal failure requiring a period of continuous renal replacement therapy (CRRT). Although pharmacokinetic studies and dosing recommendations for CMS in critically ill patients with normal or impaired renal function exist, there are only a few systematic studies of the pharmacokinetics of CMS and colistin in patients undergoing CRRT (10-13). Dose

recommendations in these patients therefore differ widely and there is evidence that certain recommendations might lead to under-dosing (14). CMS and colistin have molecular weights of 1750 and 1155 Dalton respectively, meaning they are small enough to pass through haemodiafiltration high flux membranes. Protein-binding of colistin is dependent on drug concentration and ranges between 10 and 50% in animals (15), which further enables the drug to be eliminated by CRRT. Similar protein binding values have been observed in critically ill patients (16). CMS and colistin are therefore filtered and dialyzed during CRRT (13). In addition, as no tubular reabsorption occurs during CRRT, colistin clearance is – as to be expected - higher than that achieved by a healthy kidney, even though filtration rates applied are usually less than half of a normal glomerular filtration rate (13).

The standard dosage of CMS for a 70 kg patient with normal renal function is 9 million international units (MU) per day, divided in 2-3 doses. In the USA, dosages are usually expressed in mg of colistin base activity, with a conversion factor of 33 mg for 1 MU, which corresponds to 80 mg of CMS. Previous studies have shown that reduced CMS doses ranging from 1.8 MU every 48 h to 0.9 MU every 8 h in patients undergoing continuous venovenous hemodiafiltration (CVVHDF) were associated with insufficient steady state colistin concentrations (C_{ssav}) ranging from 0.7 mg/L to 1.7 mg/L (10, 12, 14). These values are below the suggested target C_{ssav} of 2.0 - 2.5 mg/L for susceptible bacteria with a minimal inhibitory concentration (MIC) of <0.5 mg/L (12, 17-19). Minimum inhibitory concentrations for Gram-negative bacteria such as *Acinetobacter baumannii* and *P. aeruginosa* are >1 mg/l (both breakpoints 2 mg/l (20)).

Based on those studies, some authors suggest that a higher dosage is required to achieve therapeutic colistin concentrations in patients undergoing CVVHDF. A recent study of 8 patients undergoing CVVHDF (dialysate fluid rate 1–1.5 L/h) showed that the median peak colistin concentrations after a loading dose of 9 MU was 1.55 mg/l, and during maintenance therapy with 4.5 MU every 12 h a C_{ssav} of 1.72 mg/L was achieved (13).

These data indicate significant extracorporal clearance by CRRT and support the suggestion for higher dosages of CMS in critically ill patients undergoing CVVHDF, in order to avoid underdosing and subsequent therapeutic failure. Recent studies propose a CMS loading dose of 6-12 MU in critically ill patients in order to achieve effective colistin plasma concentrations from the first treatment day (13, 16, 19, 21). A loading dose of 9 MU has been shown to be well tolerated without significant renal or neurotoxicity in a study including 22 patients with normal renal function (21).

The main objective of our study was therefore to characterize the population pharmacokinetics of intravenous CMS and colistin in patients undergoing CRRT and receiving the standard dosage of CMS, predicted to achieve therapeutic concentrations in this situation according to current knowledge. Secondary study objectives were to assess clinical outcomes and drug-related adverse events.

Results

Eleven patients aged between 29 and 70 years were included in the study. One patient was subsequently excluded due to bleeding and multiple blood transfusions that lead to incorrect colistin plasma concentrations. This left ten patients for whom data were available for analysis. Patients 5 & 6 underwent CVVHDF; all other patients underwent CVVHD. Further patient characteristics including the CMS dosages administered and the settings of CRRT are shown in Table 1. The loading dose was sampled in 3 patients whereas in the remaining patients CMS was started or continued with a maintenance dose when colistin treatment was already initiated at inclusion of the patient.

CMS and colistin population pharmacokinetic profiles

A satisfactory description of observations was obtained by fitting the 6-compartment model described with NONMEM®. Individual curve-fittings of patients` measured CMS and colistin concentrations are shown in Figure 2 (see Fig. S1 for a logarithmic display). Sequential model building indicated that a model assuming interindividual variability in terms of volume and clearance parameters (V_{CMS}, V_{Col}, $CL_{M\ CMS}$ and $CL_{M\ Col}$), fitted the data better than a model assuming constant population values for these parameters. Conversely, the results did not show evidence for interindividual differences in sieving coefficients (S_{CMS} and S_{col}). We found no indication that age, body weight, sex or albumin had a role to play as covariates. Conversely, the inclusion of hematocrit (Ht) as a covariate affecting the efficacy of blood transfer (Q_{blood}) in and out of the filter improved the model significantly. Diagnostic plots in arithmetic and logarithmic form (Figures S2 and S3, respectively) visualized model validation.

The primary pharmacokinetic estimates corresponding to our final model, namely metabolic clearance, distribution volumes and sieving coefficients for CMS and colistin are given in Table 2, along with the maximum likelihood individual parameters corresponding to each study patient. In accordance with its physicochemical characteristics, CMS had a higher sieving coefficient and a smaller volume of distribution than colistin (Table 2).

The primary pharmacokinetic parameters enabled the calculation of derived parameter values such as cumulative areas und the curve, fractions excreted through CRRT, total clearances and steady-state trough concentrations of both CMS and colistin (Table 3). CMS-had a greater clearance (both total and through CVVHD) than colistin, with consequent longer colistin half-life and higher steady-state trough concentrations. CMS was more efficiently removed by CVVHD than colistin, as reflected by CRRT clearance accounting for on average 41% of the total CMS and 28% of the total colistin clearance, respectively (Table 3).

Target colistin C_{ssav} of 2.5 mg/L was achieved in all patients who received a maintenance dose of 3 MU but in none of the patients who received a maintenance dose of 2 MU (Table 3).

Adverse Events

Overall, no alarming drug accumulation was observed over the five treatment days (Figure 2 - one exception being possibly Patient 8) and no adverse events attributable to CMS or colistin were recorded.

Patient outcomes

Infection control (as evidenced by a reduction in inflammatory markers such as C-reactive protein and leucocyte count) was achieved in three patients and microbial cure in one patient. However, 7 of the 10 included patients experienced a fatal outcome of their severe illness. Renal function improved in 4 patients (moderate renal impairment, no renal replacement therapy) and one patient remained on intermittent hemodialysis.

Discussion

In a population pharmacokinetic study of intravenous CMS in patients undergoing CRRT, we found that a compartmental model incorporating CRRT settings best described the disposition of CMS and colistin. This population analysis revealed a fair degree of interindividual variability in volume and clearance parameters (V_{CMS}, V_{Col}, $CL_{M\ CMS}$ and $CL_{M\ Col}$), as is often found for therapeutic drugs undergoing metabolic biotransformations. This variability was not significantly explained by age, body weight, sex and serum albumin concentration, as these covariates did not improve model performance. However, hematocrit improved the model significantly, a finding consistent with its direct impact on drug transfer from the patient's body to the CRRT filter cartridge. It is also remarkable to observe that the model did not indicate between-patient variability in either sieving coefficients of CMS and colistin. This is not surprising for CMS, a highly hydrophilic prodrug that is poorly bound to plasma proteins. As our patients had little heterogeneity regarding their circulating albumin concentrations (Table 1), no effect of this covariate on colistin sieving could be observed, even though colistin is more extensively protein-bound than CMS. We propose that our findings will enable future optimal CMS dosing in patients

undergoing CRRT to achieve adequate antibacterial efficacy, to avoid the emergence of colistin-resistant organisms (22) and to minimize toxicity.

In their population pharmacokinetic study of CMS in critically ill patients not undergoing CRRT, Karaiskos and colleagues similarly found that a model with several compartments (5 in total) best described their data (5).

In patients with normal renal function, renal clearance accounts for two-thirds of CMS clearance and almost none of colistin clearance (3, 4). By comparison, in the present study CRRT clearance accounted for 41% of CMS clearance and 28% of colistin clearance. In the study performed by Karaiskos, CVVHDF clearance of CMS and colistin were estimated at 60% and 62% respectively (13). The lower clearance found in our study may be related to the lower blood flows (100 – 127 ml/min compared to 100 – 180 ml/min). The significant colistin clearance during CVVHDF is attributable to the absence of tubular reabsorption. In other words, while CMS clearance is reduced in renal impairment managed with CRRT, colistin clearance is increased. Consequently, higher doses of intravenous CMS are required, as already noted in some current guidelines such as the Sanford Guide to Antimicrobial Therapy, which recommends a daily CMS dose of 13 MU (442 mg colistin base) (23), but not in the Food and Drug Administration approved drug label (8).

Target colistin C_{ssav} of 2.5 mg/L for susceptible bacteria with a minimal inhibitory concentration (MIC) of <0.5 mg/L (16) was achieved in all patients who received a maintenance dose of 3 MU but in none of the patients who received a maintenance dose of 2 MU (Table 3). In the study by Karaiskos and colleagues in which 4.5 MU were administered every 12 hours, 24 hours after receiving a 9 MU loading only half achieved steady-state colistin concentrations 6 h post dose of 1.72 mg/l or higher

(13). A reason for this discrepancy is the higher CVVHDF clearance in the above-mentioned study. A further explanation might be that the 6-h post dose concentration as measured by Karaiskos and colleagues does not reflect the peak concentration attained before the next CMS dose as accurately as the pre-dose sample which was analyzed in our study (8-h post-dose).

In the study by Nation and colleagues, which included data from 9 patients undergoing CRRT (average blood flow 160 ml/min, 9 MU CMS loading dose and 13 MU daily maintenance dose) (19), CMS clearance due to CRRT was 1.57 L/h, which was identical to our finding. Colistin CRRT clearance, however, was higher (2.68 L/h, compared to 0.796 L/h in our study). In the above-mentioned study by Karaiskos and colleagues of 8 patients undergoing CRRT (blood flow 100 – 180 ml/min, 9 MU CMS loading dose and 9 MU daily maintenance dose), CMS and colistin CRRT clearances were 1.17 L/h and 2.09 L/h respectively (13). Taken together, these findings suggest that CMS-clearance may be filter-limited, while Colistin-CRRT clearance depends on blood-flow. Two other studies both using 9 MU daily maintenance doses and a blood flow of 120 ml/min (comparable to the present study) in seven and four patients undergoing CRRT (11, 24) showed colistin CRRT clearances of about 0.7 L/h, which compared to our findings.

For daily clinical practice, we therefore suggest starting CMS in patients that are undergoing CVVHD using low blood flows with a loading dose of 9 MU and a maintenance dose of 3 MU every 8 hours (independent of body weight), so that therapeutic colistin concentrations have a high likelihood of being achieved. As $C_{ss\,av}$ only depends on clearance (and not volume of distribution), the maintenance dose does not need to be weight-adjusted.

The present study has some limitations. Although prospective in design, patients were included based on their clinical need for intravenous CMS and concurrent CRRT. This resulted in a modest sample size and inability to perform PK-sampling after a loading dose in the setting of CRRT in every case. Despite satisfactory global diagnostics (Fig. S2 and S3 in supplementary material), isolated misfit points are observed, most likely resulting from technical issues such as inaccuracies in dose preparation, sample collection, or CVVHD efficacy due to filter clogging or clotting. A further limitation is the missing data regarding hematocrit. However, the available data showed a small spread, so we are confident that using the average value for the group as a whole did not significantly affect the results.

In conclusion, CMS and colistin disposition after intravenous application is complex and CRRT substantially increases the clearance of colistin compared to normal renal function. A loading dose of 9 MU and a maintenance dosage of 3 MU every 8 hours independent of body weight are expected to quickly achieve and maintain therapeutic colistin concentrations in patients undergoing CRRT with low blood flows. We suggest starting the maintenance dosage 8 hours after administering the loading dose. Furthermore, this dosage was not associated with adverse events. Therapeutic drug monitoring of CMS and colistin might further ensure optimal dosing, particularly in problematic situations such as co-morbid hepatic insufficiency or inefficient CRRT.

Materials and Methods

The study was performed in accordance with the Declaration of Helsinki and its amendments, International Conference on Harmonization (ICH) Good Clinical

Practice (GCP) guidelines and applicable national laws and regulations. Ethical approval for the study was granted by the internal review boards of the participating centers (ZH 2012-0451, CER-VD 197/14 and EKNZ 2014-213) and permission to conduct the study was given by the national authority (Swissmedic, 2013DR1132). The study was registered at ClinicalTrials.gov (NCT02081560). All patients enrolled in the study gave their written, informed consent if able to do so. When patients were not able to give consent themselves, the protocol for studies in adult patients unable to give consent was followed, in accordance with the internal review boards' permission.

Study design

The study is an investigator-driven, multi-center, prospective, non-randomized, open label pharmacokinetic investigation of CMS in critically ill patients requiring CVVHDF. The participating centers were University Hospital Zurich, University Hospital Lausanne and University Hospital Basel.

Study patients

Patients were eligible for enrolment if they fulfilled the following criteria: male or female aged 18 years or older, hospitalized on the intensive care unit (ICU) with a clinical necessity for both, treatment with CMS (for a proven severe infection with multi drug resistant Gram-negative bacteria susceptible to colistin with no standard first-line treatment available) and continuous venovenous renal replacement therapy.

Study drug

Colistin® (colistimethate) was delivered in dry-powder form together with an ampoule of 3 ml isotonic saline solvent (1). One bottle of Colistin® contains 1 million units

(MU) of sodium colistimethate. Colistin® was dispensed from the local hospital pharmacy and stored on the intensive care units. CMS maintenance dosing was set to 3 MU (=240 mg) every 8 hours (total 9 MU/d = 720 mg/d) after administration of a loading dose of 9 MU. In patients with a low body weight (≤ 60 kg), the dose was reduced to a loading dose of 6 MU and to a maintenance dose of 2 MU every 8 hours. The CMS solution was to be administered intravenously over 30 minutes. For pharmacokinetic calculations, CMS doses in mg and concentrations in mg/L were converted into colistin equivalents using a conversion factor of 1155/1750 = 0.66.

CRRT procedures

Continuous venovenous hemodialysis (CVVHD) was performed in all but one patient with Prismaflex ST150 (Gambro AB, Lund, Sweden) using the capillary hemofilter AN69 ST (acrylonitrile-sodium-methyl sulfonate, surface area 1.5 m^2) and infusing citrate as the anti-coagulation agent. Continuous venovenous hemodiafiltration (CVVHDF) in a ratio set 1:1 was performed with Multifiltrate (Fresenius Medical Care, Homburg, Germany) using the capillary hemofilter AV 1000s (polysulfone, surface area 1.8 m^2). Post-dilution mode was performed for one patient.

Pharmacokinetic sampling

Intensive pharmacokinetic (PK) sampling over one dosing interval (8 hours) was scheduled on treatment day 1 after the CMS loading dose and day 3 (after the 7th dose). Intensive PK sampling involved blood samples taken from the pre-filter ("filter afferent") line at 0, 0.5, 1, 2, 3, 6, and 8 hours after CMS dosing and from the post-filter line at 1 and 8 hours after dosing. Dosing and sampling times were precisely recorded, along with the relevant settings of CRRT (blood and hemofiltration flow

rates). Blood was collected in tubes containing citrate. Plasma was obtained by centrifugation of whole blood samples within 30 minutes and then stored at -80°C until analysis to prevent degradation of CMS into colistin ex-vivo (25). Peak and trough blood concentrations in the pre-filter line were determined on day 5. Patients already on colistin treatment at study entry underwent PK sampling on treatment day 3 (7th dose) and on day 5 if already undergoing CRRT. In patients starting CRRT during established treatment with Colistin®, the dosage was adapted according to the protocol, the first PK sampling was performed on the third day of CRRT, and the study continued according to the protocol. Patients were monitored daily for any abnormalities in blood biochemistry and hematology values and for signs of adverse drug effects.

Determination of plasma CMS and colistin concentrations

Plasma CMS and colistin concentrations were determined by high-throughput hydrophilic interaction chromatography coupled to tandem mass spectrometry according to the method described by Mercier and colleagues (26). Briefly, the method was first applied to the native plasma samples to determine colistin concentration; then the samples underwent acidic hydrolysis for 4h to extensively transform CMS into colistin, colistin was determined again and the difference was assumed to reflect CMS (including intermediate metabolites partially hydrolyzed). The results of CMS were accordingly expressed in mg/L of colistin. All analyses were performed at the University Hospital Lausanne.

Pharmacokinetic analysis

318 A model-based pharmacokinetic analysis was performed using NONMEM® (version
319 7.4, 2017, ICON plc, Dublin 18, Ireland) with NMTRAN and the PREDPP subroutine
320 ADVAN6. The disposition of CMS and colistin was described using a 6-compartment
321 model with first-order transfer rates describing the metabolic transformation of CMS
322 into colistin, the metabolic elimination of colistin and the exchanges of both
323 compounds occurring within the CRRT apparatus (Fig. 1). The systemic distributions
324 of CMS and colistin were assumed to be rapid, corresponding to a single-
325 compartment volume each (V_{CMS} and V_{Col}). The fraction of the CMS dose escaping
326 elimination through CRRT was assumed to be completely transformed into colistin
327 through metabolic clearance ($CL_{M\ CMS}$); then colistin, in turn, was considered to be
328 eliminated through both CRRT and metabolic clearance ($CL_{M\ Col}$). Both compounds
329 were assumed to be transferred into a CRRT filter of fixed volume (V_{filter} = 0.2 L) at
330 the flow rate read on the CRRT device (Q_{blood}), corrected for the patient's hematocrit
331 (1 - Ht), set to the average HT value (0.25) when unknown. Non-filtered amounts of
332 CMS and colistin were driven back into the patient's circulation with the same blood
333 flow, while filtered amounts passed into the fixed volume filter cartridge ($V_{cartridge}$ = 0.3
334 L), continuously rinsed with the effluent flow rate read on the CRRT device (Q_{effl}).
335 Sieving coefficients were assumed to characterize the filter permeability for CMS and
336 colistin (S_{CMS} and S_{col}), multiplying Q_{effl} to give the respective filtration clearances.

337 The model, expressed as a set of differential equations, was fitted to the data using
338 first order conditional estimation with interaction between inter-patient and residual
339 variabilities. Classical equations were used for relating model microconstants (k_{12},
340 k_{13}, k_{31}, k_{20}, k_{24}, k_{42}, k_{35}, k_{46}, k_{50} and k_{60}) to the estimated parameters, i.e. V_{CMS}, V_{Col},
341 $CL_{M\ CMS}$, $CL_{M\ Col}$, S_{CMS} and S_{col}, given the known parameters, i.e. V_{filter}, $V_{cartridge}$, Q_{blood},

Ht and Q_{effl}. Interindividual variability in estimated parameters was described using an exponential error model (namely $\theta_j = \theta \cdot e^{\eta_j}$, where θ_j is the individual pharmacokinetic parameter of the j^{th} individual, θ is the geometric average population value and η_j is a random effect normally distributed around a zero mean with a variance of ω^2_θ). The inclusion of variability was tested on each parameter for improvement of the model, based on the change in objective function (ΔOF) resulting from the addition of a model term (which approximately follows a χ^2 distribution and can be regarded as statistically significant at the level $p<0.05$ if it exceeds 3.8 for one additional parameter). Residual error was described using a mixture of exponential plus additive distributions; there was no indication for different magnitudes of variability between the types of samples. The influence of covariates such as age, sex and body weight on distribution volumes and metabolic clearances, and plasma albumin on sieving coefficients was explored graphically and tested sequentially for inclusion in the model, based on the ΔOF criterion. The following derived parameters were computed numerically from individual parameter estimates obtained by model fitting: cumulative areas under plasma concentration curves (AUC_{CMS} and AUC_{Col}), trough concentrations at steady-state under maintenance dosage ($C_{SS\ min\ CMS}$ and $C_{SS\ min\ Col}$) and total amounts excreted by CRRT ($A_{e\ CMS}$ and $A_{e\ Col}$). Taking into account cumulative CMS doses, we estimated the fractions of dose eliminated by CRRT ($F_{e\ CMS}$ and $F_{e\ Col}$), the hemofiltration ($CL_{CRRT\ CMS}$ and $CL_{CRRT\ Col}$) and total clearances ($CL_{tot\ CMS}$ and $CL_{tot\ Col}$), and the apparent half-lives deduced from corresponding distribution volumes ($t_{½\ CMS}$ and $t_{½\ Col}$).

Model validation was based on diagnostic goodness-of-fit plots and on the examination of standard errors and correlation matrix of the estimates. Figures were

366 elaborated with GraphPad Prism (Version 7 for Windows, 2017, GraphPad Software,
367 San Diego California USA). The population pharmacokinetic model developed for this
368 study may be accessed on the DDMoRe Model Repository at:
369 http://repository.ddmore.foundation//model/DDMODEL00000295.

370

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377 **References**

378 1. Product Information Colistin(R). 2016. Teva Pharma AG, Basel, Switzerland.
379 2. Bergen PJ, Li J, Rayner CR, Nation RL. 2006. Colistin methanesulfonate is an
380 inactive prodrug of colistin against Pseudomonas aeruginosa. Antimicrob
381 Agents Chemother 50:1953-8.
382 3. Gregoire N, Aranzana-Climent V, Magreault S, Marchand S, Couet W. 2017.
383 Clinical Pharmacokinetics and Pharmacodynamics of Colistin. Clin
384 Pharmacokinet 56:1441-1460.
385 4. Couet W, Gregoire N, Gobin P, Saulnier PJ, Frasca D, Marchand S, Mimoz O.
386 2011. Pharmacokinetics of colistin and colistimethate sodium after a single 80-
387 mg intravenous dose of CMS in young healthy volunteers. Clin Pharmacol
388 Ther 89:875-9.
389 5. Karaiskos I, Friberg LE, Pontikis K, Ioannidis K, Tsagkari V, Galani L,
390 Kostakou E, Baziaka F, Paskalis C, Koutsoukou A, Giamarellou H. 2015.
391 Colistin Population Pharmacokinetics after Application of a Loading Dose of 9
392 MU Colistin Methanesulfonate in Critically Ill Patients. Antimicrob Agents
393 Chemother 59:7240-8.
394 6. Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, Silveira
395 FP, Forrest A, Nation RL. 2011. Population pharmacokinetics of colistin
396 methanesulfonate and formed colistin in critically ill patients from a multicenter

study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother 55:3284-94.

7. Goodwin NJ, Friedman EA. 1968. The effects of renal impairment, peritoneal dialysis, and hemodialysis on serum sodium colistimethate levels. Ann Intern Med 68:984-94.

8. Coly-Mycin® M Parenteral (Colistimethate for Injection, USP). 2017. Par Pharmaceutical, Chestnut Ridge, NY 10977.

9. Falagas ME, Kasiakou SK. 2006. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. Critical Care 10.

10. Karvanen M, Plachouras D, Friberg LE, Paramythiotou E, Papadomichelakis E, Karaiskos I, Tsangaris I, Armaganidis A, Cars O, Giamarellou H. 2013. Colistin methanesulfonate and colistin pharmacokinetics in critically ill patients receiving continuous venovenous hemodiafiltration. Antimicrob Agents Chemother 57:668-71.

11. Leporati M, Bua RO, Mariano F, Carignano P, Stella M, Biancone L, Vincenti M. 2014. Determination by LC-MS/MS of colistins A and B in plasma and ultrafiltrate from critically ill patients undergoing continuous venovenous hemodiafiltration. Ther Drug Monit 36:182-91.

12. Markou N, Fousteri M, Markantonis SL, Zidianakis B, Hroni D, Boutzouka E, Baltopoulos G. 2012. Colistin pharmacokinetics in intensive care unit patients on continuous venovenous haemodiafiltration: an observational study. J Antimicrob Chemother 67:2459-62.

13. Karaiskos I, Friberg LE, Galani L, Ioannidis K, Katsouda E, Athanassa Z, Paskalis H, Giamarellou H. 2016. Challenge for higher colistin dosage in critically ill patients receiving continuous venovenous haemodiafiltration. Int J Antimicrob Agents 48:337-41.

14. Li J, Rayner CR, Nation RL, Deans R, Boots R, Widdecombe N, Douglas A, Lipman J. 2005. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. Antimicrobial Agents and Chemotherapy 49:4814-4815.

15. Dudhani RV, Turnidge JD, Coulthard K, Milne RW, Rayner CR, Li J, Nation RL. 2010. Elucidation of the pharmacokinetic/pharmacodynamic determinant of colistin activity against Pseudomonas aeruginosa in murine thigh and lung infection models. Antimicrob Agents Chemother 54:1117-24.

16. Mohamed AF, Karaiskos I, Plachouras D, Karvanen M, Pontikis K, Jansson B, Papadomichelakis E, Antoniadou A, Giamarellou H, Armaganidis A, Cars O, Friberg LE. 2012. Application of a loading dose of colistin methanesulfonate in critically ill patients: population pharmacokinetics, protein binding, and prediction of bacterial kill. Antimicrob Agents Chemother 56:4241-9.

17. Pogue JM, Ortwine JK, Kaye KS. 2017. Clinical considerations for optimal use of the polymyxins: A focus on agent selection and dosing. Clin Microbiol Infect 23:229-233.

18. Nation RL, Garonzik SM, Li J, Thamlikitkul V, Giamarellos-Bourboulis EJ, Paterson DL, Turnidge JD, Forrest A, Silveira FP. 2016. Updated US and

European Dose Recommendations for Intravenous Colistin: How Do They Perform? Clin Infect Dis 62:552-558.

19. Nation RL, Garonzik SM, Thamlikitkul V, Giamarellos-Bourboulis EJ, Forrest A, Paterson DL, Li J, Silveira FP. 2017. Dosing guidance for intravenous colistin in critically-ill patients. Clin Infect Dis 64:565-571.

20. Anonymous. www.eucast.org, last accessed July 2017.

21. Dalfino L, Puntillo F, Mosca A, Monno R, Spada ML, Coppolecchia S, Miragliotta G, Bruno F, Brienza N. 2012. High-dose, extended-interval colistin administration in critically ill patients: is this the right dosing strategy? A preliminary study. Clin Infect Dis 54:1720-6.

22. Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A, 3rd, Forrest A, Bulitta JB, Tsuji BT. 2010. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. Pharmacotherapy 30:1279-91.

23. Sanford Guide to Antimicrobial Therapy Web Edition, Antimicrobial Therapy, Inc. Last accessed 3rd April 2018.

24. Mariano F, Leporati M, Carignano P, Stella M, Vincenti M, Biancone L. 2015. Efficient removal of colistin A and B in critically ill patients undergoing CVVHDF and sorbent technologies. J Nephrol 28:623-31.

25. Dudhani RV, Nation RL, Li J. 2010. Evaluating the stability of colistin and colistin methanesulphonate in human plasma under different conditions of storage. J Antimicrob Chemother 65:1412-5.

26. Mercier T, Tissot F, Gardiol C, Corti N, Wehrli S, Guidi M, Csajka C, Buclin T, Couet W, Marchetti O, Decosterd LA. 2014. High-throughput hydrophilic interaction chromatography coupled to tandem mass spectrometry for the optimized quantification of the anti-Gram-negatives antibiotic colistin A/B and its pro-drug colistimethate. J Chromatogr A 1369:52-63.

Table 1

Patient description: age, body weight, sex, loading dose, maintenance dose, number of maintenance doses administered, average blood flow and effluent rate through CRRT device. Alb.: albumin, CV: coefficient of variation, CVVHD: continuous venovenous hemodialysis, CVVHDF: continuous venovenous hemodiafiltration, D_{load}: CMS loading dose, D_{maint}: CMS maintenance dose, D_{maint} tot : CMS total number of maintenance doses, gmean: geometric mean, Hct: hematocrit, MIC: minimum inhibitory concentration (ranges indicate results of different isolates tested over time), na: not available, Q_{blood} : blood flow, Q_{effl}, effluent flow rate, VAP: ventilator-associated pneumonia

ID	Age (y)	Wt (kg)	Sex	Diagnoses and indication for colistin	Colistin MICs	Hct	Alb (g/l)	D_{load} (MU)	D_{maint} (MU)	D_{maint} tot (MU)	CRRT	Q_{blood} (mL/min)	Q_{effl} (L/h)	Outcome
1	60	65	M	Acute lymphoblastic leukaemia, pneumonitis, *P. aeruginosa* bacteraemia	<4	na	na	9	3	18	CVVHD	100	2180	Infection controlled
2	51	73	M	Sepsis with multiresistant *P. aeruginosa*, multiorgan failure	na	na	na	9	3	7	CVVHD	100	2000	Died (multiorgan failure)
3	29	82	M	Polytrauma, *P. aeruginosa* pneumonia and septic shock	0.75-1	0.24	22	9	3	15	CVVHD	108	2793	Infection controlled
4	44	60	M	Lung transplant, mediastinal abscess, acute respiratory failure (*P. aeruginosa* VAP)	0.38	0.33	20	2	2	14	CVVHD	122	3071	Infection controlled
5	34	60	M	Cycstic fibrosis, liver cirrhosis, *P. aeruginosa* pneumonia and septic shock	0.047-0.5	0.27	28	2	2	24	CVVHDF	120	2479	Died (pulmonary hemorrhage)
6	47	66	M	HIV, pulmonary hypertension, PCP pneumonia, acute respiratory distress syndrome, *P. aeruginosa* and *Citrobacter freundii* VAP	1 0.125	0.23	21	9	3	12	CVVHDF	127	2473	Died (respiratory failure)
7	67	60	M	Cholangitis, septic shock (*E.coli* ESBL), acute respiratory distress syndrome	na	0.26	22	6	2	6	CVVHD	118	2250	Died (cholangitis)
	70	85	M	Pancreas carcinoma, post-operative septic shock, *P. aeruginosa* VAP	1.5	na	na	3	3	19	CVVHD	100	2278	Died (multiorgan failure)
10	44	46	F	Lung transplant, *P. aeruginosa* tracheobronchitis	0.125	0.23	20	2	2	22	CVVHD	80	1601	Died (cause unknown) after Infection cleared
11	68	60	F	Necrotising pancreatitis, ventilator-associated pneumonia, severe sepsis	na	0.25	24	9	3	10	CVVHD	100	2652	Died (multiorgan failure)
Summary statistics														
mean	50.4	65.7										108	2378	
SD	16.0	11.6										14	416	
median	49.0	62.5										104	2376	
min	24.0	46.0										80	1601	
max	70.0	85.0										127	3071	
gmean	47.8	64.8										107	2343	
CV	34%	18%										13%	18%	

477 **Table 2**

478 Population averages and coefficients of variation (CV), followed by maximum
479 likelihood individual estimates of primary pharmacokinetic parameters: metabolic
480 clearances, distribution volumes and sieving coefficients for CMS and colistin.
481 Residual error is described by a mixed exponential and additive error model, with
482 respectively a coefficient of variation of 22.2% and a standard deviation (SD) of 0.459
483 mg/L. $CL_{M\ CMS}$: metabolic clearance of CMS, V_{CMS}: volume of distribution of CMS, S_{CMS}: CMS
484 sieving coefficient, $CL_{M\ Col}$: metabolic clearance of colistin, V_{Col}: volume of distribution of
485 colistin, S_{Col} Colistin sieving coefficient, Pop: population SE: standard error, geomean:
486 geometric mean.

		CMS			Colistin		
		$CL_{M\ CMS}$ (L/h)	V_{CMS} (L)	S_{CMS}	$CL_{M\ Col}$ (L/h)	V_{Col} (L)	S_{Col}
Pop estimate		2.31	12.1	1.05	1.93	70.1	0.454
SE		0.412	1.42	0.0667	0.444	15.5	0.0181
Pop CV		52%	36%	-	67%	50%	-
SE		0.162	0.106	-	0.218	0.127	-
Patient estimates							
	1	2.34	12.2	1.050	3.18	35.5	0.454
	2	7.33	28.3	1.050	2.50	69.0	0.454
	3	2.41	11.7	1.050	1.81	84.8	0.454
	4	1.98	8.9	1.050	2.01	85.4	0.454
	5	2.41	11.9	1.050	2.14	73.3	0.454
	6	3.12	14.8	1.050	1.54	67.6	0.454
	7	1.62	8.0	1.050	2.49	70.8	0.454
	8	1.46	9.6	1.050	1.38	123.6	0.454
	10	2.79	10.1	1.050	4.09	73.1	0.454
	11	1.13	13.4	1.050	0.54	39.0	0.454
mean		2.66	12.9	1.050	2.17	72.2	0.454
SD		1.75	5.8		0.99	24.6	
median		2.37	11.8		2.07	72.0	
min		1.13	8.0		0.54	35.5	
max		7.33	28.3		4.09	123.6	
geomean		2.32	12.1		1.93	68.3	
CV		75%	48%		51%	36%	

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Table 3

Secondary pharmacokinetic parameters derived from individual estimates of Table 2: cumulative areas under curve (AUC), fractions excreted through CRRT (F_e), total clearances (CL_{tot}), trough concentrations at steady-state ($C_{ss\ min}$) and average concentrations at steady-state ($C_{ss\ av}$) of CMS and colistin. SD: standard deviation, CV: coefficient of variation. For dose-dependent parameters (AUC and $C_{SS\ min}$), means, SD, medians and ranges were calculated only with data from patients receiving a maintenance regimen of 3 MU every 8 h (marked by *).

ID	CMS							Colistin						
	AUC_{CMS} (h·mg/L)	$F_{e\ CMS}$	$CL_{tot\ CMS}$ (L/h)	$CL_{CVVHD\ CMS}$ (L/h)	$t_{½\ CMS}$ (h)	$C_{SS\ min\ CMS}$ (mg/L)	$C_{SS\ av\ CMS}$ (mg/L)	AUC_{Col} (h·mg/L)	$F_{e\ Col}$	$CL_{tot\ Col}$ (L/h)	$CL_{CVVHD\ Col}$ (L/h)	$t_{½\ Col}$ (h)	$C_{SS\ min\ Col}$ (mg/L)	$C_{SS\ av\ Col}$ (mg/L)
1*	865	0.393	3.850	1.513	2.20	1.26	5.15	506	0.203	3.993	0.811	6.16	2.58	3.01
2*	181	0.163	8.766	1.429	2.24	0.58	2.26	404	0.230	3.283	0.755	14.57	4.63	5.05
3*	658	0.434	4.265	1.851	1.91	0.86	4.65	548	0.348	2.899	1.009	20.29	3.69	3.87
4	(406)	0.484	3.840	1.859	1.61	(0.34)	(3.44)	(251)	0.338	3.204	1.083	18.48	(1.91)	(2.13)
5	(621)	0.424	4.183	1.774†	1.98	(0.62)	(3.16)	(480)	0.299	3.124	0.934†	16.26	(2.28)	(2.44)
6*	479	0.361	4.889	1.765†	2.10	0.83	4.05	590	0.370	2.534	0.938†	18.47	4.64	5.00
7	(312)	0.459	2.996	1.375	1.85	(0.58)	(4.41)	(163)	0.163	3.110	0.507	15.79	(1.87)	(2.30)
8*	1044	0.511	2.990	1.528	2.24	1.60	6.63	607	0.329	2.511	0.826	34.12	3.92	3.85
10	(608)	0.290	3.933	1.141	1.78	(0.51)	(3.36)	(361)	0.128	4.702	0.602	10.78	(1.75)	(1.99)
11*	765	0.574	2.651	1.522	3.50	2.41	7.47	741	0.430	1.164	0.501	23.21	4.00	7.24
mean	665	0.409	4.236	1.576	2.14	1.26	5.03	566	0.284	3.052	0.796	17.81	3.91	4.67
SD	304	0.117	1.731	0.222	0.52	0.67	1.86	112	0.098	0.933	0.194	7.48	0.76	1.48
median	712	0.429	3.892	1.525	2.04	1.06	4.90	569	0.314	3.117	0.804	17.37	3.96	4.43
min	181	0.163	2.651	1.141	1.61	0.58	2.26	404	0.128	1.164	0.501	6.16	2.58	3.01
max	1044	0.574	8.766	1.859	3.50	2.41	7.47	741	0.430	4.702	1.083	34.12	4.64	7.24
geomean	583	0.390	4.003	1.559	2.09	1.12	4.70	557	0.266	2.894	0.771	16.35	3.84	4.49
CV	52%	30%	43%	14%	25%	60%	40%	20%	37%	32%	25%	46%	20%	33%

* 3 MU q. 8 h maintenance dose; † CL_{CVVHDF}

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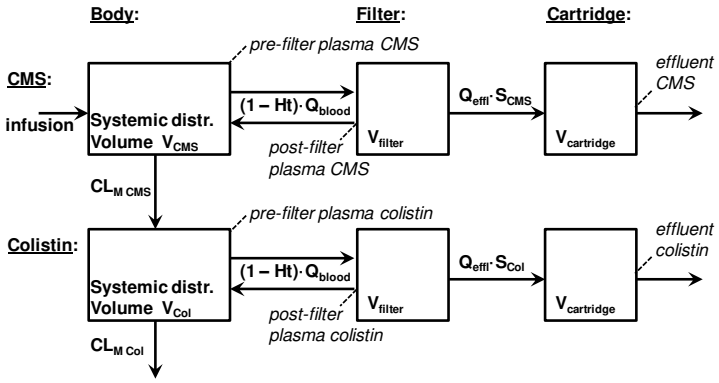
497 **Figure 1**

498 Six-compartment pharmacokinetic model describing the disposition of CMS and colistin during CRRT, and showing the sampling

499 points (in italic). See text for parameter definitions.



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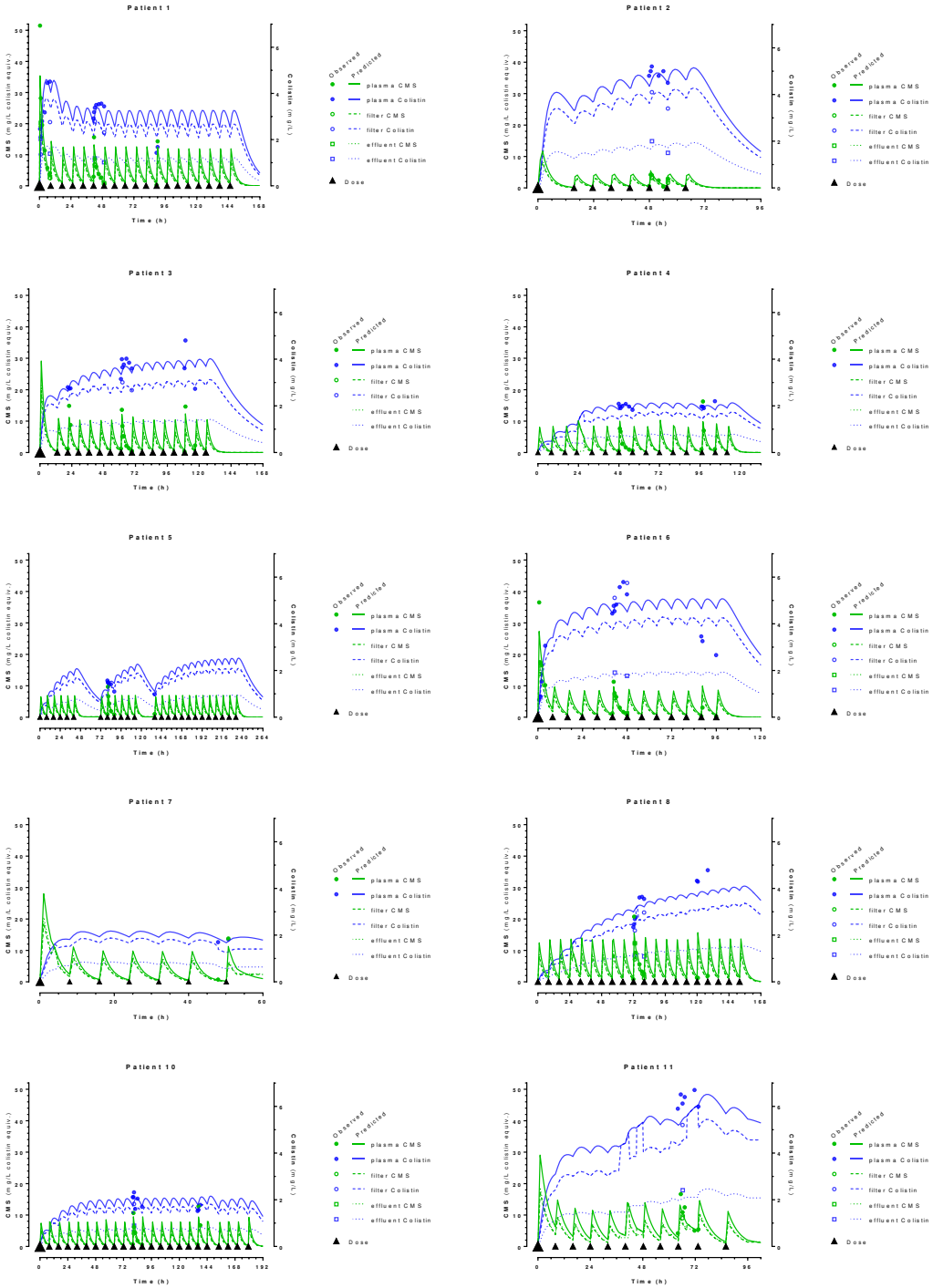
502 **Figure 2**: Individual curve fitting of patients` observations (solid circles)



503 in arithmetic scale

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